

### CONCLUSION

If any minor matters remain to be discussed prior to examination, the Examiner is invited to contact the undersigned at the telephone number listed below.

Respectfully submitted,

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(N)-terminus of full-length L1 was fused to [aminoacid] amino acid residues 50-65 from E1 protein of HPV16, and (C) the amino (N)-terminus of full-length L1 was fused to amino acid residues 50-65 from E1 protein HPV16 and additionally, [aminoacid] amino acid residues 384-403 from E1 protein of HPV16 were fused at the N-terminus corresponding to sequence (B).

For generation of recombinant (A), the sequence of HPV16 L1 ORF was amplified by polymerase chain reaction (PCR) from a total DNA extract of W1 2, an HPV16 episome-containing cell line (Stanley M, Brown HM, Appleby M, and Minson AC (1989) "Properties of a non-tumorigenic human keratinocyte cell line", Int J Cancer 43, 672-676) using a forward primer in which a Bgl 11 restriction sequence was included (underlined) 5'-GCT GCA AGA TCT ATG TCT CTT TGG CTG CCT AG-3' (**SEQ ID NO: 1**).

For generation of recombinant (B), a DNA nucleotide sequence encoding the E1 50-65 sequence plus a Bgl 11 restriction sequence (underlined) was introduced just in front of the L1 coding sequence as a forward primer, as follows: 5'-GCT GCA AGA TCT ATG GTA GAT TTT ATA GTA AAT GAT AAT GAT TAT TTA ACA CAG GCA GAA TCT CTT TGG CTG CCT AGT GAG-3' (**SEQ ID NO: 2**).

For generation of recombinant (C), a nucleotide sequence-encoding E1 amino acids 384-403 was introduced just in front of E1 50-65 coding sequence of the last-mentioned construct, using a forward primer with a flanking Bgl 11 restriction sequence (underlined): 5'-GCT GCA AGA TCT ATG TAC GAT AAT GAC ATA GTA GAC GAT AGT GAA ATT GCA TAT AAA TAT GCA CAA TTG GCA GAC GTA GAT TTT ATA GTA AAT GAT-3' (**SEQ ID NO: 3**).

These forward primers were paired with the same reverse primer in which a Not 1 restriction site was included (underline). Reverse: 5'-GAT CTA GCG GCC GC TTA CAG CTT ACG CTT CTT GCG TTT-3' (**SEQ ID NO: 4**).

Following 30 cycles of amplification, the DNA products (e.g. of about 1.7kb in size) were gel purified, GENECLAN (TM) excised, digested with restriction enzymes of Bgl 11 and Not 1 and sub-cloned into baculovirus transfer vector pBacAK8 (Clontech) which had been pre-digested with BamHI and Not I restriction enzymes. The recombinant plasmids for cases (A), (B) and (C) were examined by sequencing in per-se known manner (Pharmacia (TM) kit).

#### **Generation of recombinant baculoviruses:**

Insect cells of *Spodoptera frugiperda* (sf21) were grown in 30 mm dishes until 80% confluent, at 27°C with TNMFH medium (Sigma) supplemented with 10% foetal